The Chiral Bilayer Effect Stabilizes Micellar Fibers

Fuhrhop, Jürgen-Hinrich; Schnieder, Peter; Rosenberg, Jörg; Boekema, Egbert

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Supplementary Material Available: General experimental section; IR, MS, NMR, and/or UV data for compounds **1–26**, **29–32**, **35–38**, **40**, **42–47**, **49–65**, 4-bromo-2-nitrotoluene, and 6-bromoindole-3-glyoxylamide; Table I, UV Data for Eudistomins and Their Derivatives; Table II, ^{1}H NMR Data for Eudistomins and Their Derivatives; Table III, ^{13}C NMR Chemical Shifts of **1**, **7–9**, **64**, and **65**; Figure 1, COSY of Diacetyleudistomin E (**65**); Figure 2, CD Spectra of Diacetyleudistomins C and E (**64** and **65**); and an isolation flow chart of *E. olivaceum* (22 pages). Ordering information is given on any current masthead page.

The Chiral Bilayer Effect Stabilizes Micellar Fibers

Jürgen-Hinrich Fuhrhop,*† Peter Schnieder,†‡ Jörg Rosenberg,† and Egbert Boekema‡

Contribution from the Institut für Organische Chemie der Freien Universität, Berlin, 1000 Berlin 33, West Germany, and Fritz-Haber-Institut der Max-Planck Gesellschaft, Faradayweg 4-6, 1000 Berlin 33, West Germany. *Received April 24, 1986*

Abstract: Dihelical fibers several micrometers in length and gels were obtained by spontaneous aggregation of octyl L- and D-gluconamides. The single strands have the thickness of a bimolecular layer. No fibers are formed from the racemate. The tendency of the chiral amphiphiles to aggregate to very long fibers instead of three-dimensional crystals is rationalized with a "chiral bilayer effect". This effect is caused by the slowness of rearrangements from tail-to-tail hydrophobic bilayers to crystals, in which the molecular sheets are arranged in a head-to-tail fashion. Thermograms which indicate slow rearrangements in ageing gels are also reported.

The hydrophobic effect[1] impresses sheet-like bilayer structures on the aggregates of water-insoluble amphiphile molecules of cylindrical shape.[2,3] These bilayers may align to form myelin figures[4] or rearrange to spherical vesicle membranes.[5] If the head groups of amphiphiles (i) are chiral and (ii) contain an amide bond, helical fibers and gels may be formed from vesicular or micellar solutions.[6,7] It appears that the formation of essentially linear hydrogen bonds between the amide groups is responsible for this rearrangement.[7] Arnett and Thomson have demonstrated enantiomer discrimination in two-dimensional monolayers of chiral stereamides.[8] In organic solvents helical aggregates of chiral non-amide amphiphiles have also been observed.[9,10] In these cases the corresponding racemate did not produce fibers but platelets.

We are interested in linear aggregates in aqueous media, because they constitute the structural counterpart to spherical vesicles. A combination of both may produce vesicle membranes with protrusions and/or ordered gel structures in the inner volume. This is considered as an important synthetic step toward functional cell models.[3]

In this paper we describe a new type of "bulgy double helix" made from *N*-alkylgluconamides in water. The single strands of these helices are as thin as molecular bilayers, which can only be arranged in tail-to-tail fashion. Anhydrous crystals of *N*-alkyl-D-gluconamides, however, show head-to-tail (or enantiopolar) packings of adjacent molecular sheets.[11] This structural phenomenon is used to introduce a new "chiral bilayer effect", which explains the longevity of chiral fibers and gels as compared to racemic analogues, which precipitate as crystals.

Experimental Section

Syntheses of Gluconamides. The D-gluconamides **1a** and **1b** were obtained by aminolysis of D-glucono-δ-lactone (Sigma, Deisenhofen) with *n*-octylamine or *n*-docecylamine in methanol. L-Glucono-γ,δ-lactones were prepared by indirect electrolytic oxidation in the presence of calcium bromide and calcium carbonate.[12,13] Excess bromide was precipitated with silver carbonate. The filtered solution was treated with a strongly acidic ion exchange resin (Merck) and dehydrated by azeotropic destillation with 1-butanol. The yield of L-glucono-γ,δ-lactones was 80%, the final aminolysis with octylamine occurred quantitatively. mp (**1a**) = 158°C, (**2**) 156°C, **1a+2** (1:1) 154.5°C; $[a]^{25}D$(1a) = +28.3° (in Me_2SO); $[\alpha]^{25}_D$(**2**) = −27.1° (in Me_2SO); spectra (IR, ^{1}H NMR, and MS) and elemental analyses (C, H, N) are added as supplementary material.

Gels and Electron Micrographs. Gels were formed by heating **1a** or **1b** or **2** in water to 100 °C and cooling to room temperature. They were obtained in the concentration range from 0.5 to 50% (w/v). Below 0.5% incoherent gel flakes in fluid water were formed. Above 50% the solution remained turbid at 100 °C. The gels remained clear for a few hours. After a day crystals began to separate.

At pH 2 and in the presence of 2% phosphotungstic acid, however, 2–20% (w/v) gels remained clear for weeks. This behavior has also been observed for polysaccharide gels.[14] Electron microscopy was carried out with a Philips EM 300 at 80 kV and direct magnification of 70 000.

Negatively stained samples were prepared by dipping carbon-coated

* Address correspondence to this author.
† Institut für Organische Chemie der Freien Universität.
‡ Fritz-Haber-Institut der Max-Planck Gesellschaft.

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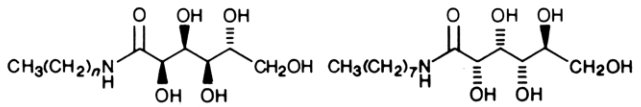
grids into fresh 1% (w/v) gels containing 2% (w/v) phosphotungstic acid (PTA). The pH was adjusted to 7 with sodium hydroxide.

Some samples were additionally shadowed with platinum–carbon at an elevation angle of 30° with use of Edwards Coating System E 306 A. The exposure of the shadowed surfaces to the electron beam had to be carried out very carefully. Image processing was performed on a VAX 780 computer using the IMAGIC software.[15]

Differential Scanning Calorimetry. A Perkin-Elmer DSC-2C calorimeter was used. The dry samples (2–4 mg) were weighed into large pans, 65 μL of water was added, and the pans were sealed. The pans were then heated to 100 °C for a few minutes and cooled to room temperature to prepare the gels. After this pretreatment the gels were again heated to 100 °C and then cooled and reheated at a rate of 2.5 deg/min. "Aged gels" were obtained by leaving the sample at room temperature overnight. The enthalpy changes were determined by measuring the peak area of the thermogram. The accuracy ranges for T_m and ΔH were ±0.5 K and ±2 kJ/mol.

Results and Discussion

Molecular Bilayer Cylinders. Aqueous gels from the D-gluconamides **1a,b** have already been obtained by Pfannemüller and Welte.[7] Their electron micrographs revealed regular helical ropes or rods of diameters between 200 and 300 Å. We reproduced their structures and also prepared twisted ribbons of the octadecyl derivative **1c** in Me_2SO, as well as multilayered tubes of **1a** in xylene.[16]



1a, $n = 7$
1b, $n = 11$
1c, $n = 17$

2

The number of the helical strands and the orientation of individual molecules can, however, not be deduced from these structures.

Since the hydrophobic effect appeared to be the only possible driving force to yield the helices, we searched for micellar cylinders of the ultimate molecular bilayer thinness. This would be about 35 Å for the octylamide **1a** and 45 Å for the dodecylamide **1b**. Both cylinders were finally realized (see Experimental Section) in the form of "bulgy double-helices". They consist of two strains which form wide bulges and knots at regular distances of 238 Å (Figure 1a,c). Besides these bulgy helices we also observed twisted ribbons (Figure 1b). Noise reduced computer images of the octyl- and dodecylamide "bulgy helices" (Figure 3c,d) allowed measurements of the knot diameters. They were 7.0 ± 0.3 and 9.0 ± 0.3 nm, corresponding to the lengths of four molecules. The single strands of the bulgy helices were also twisted (see Figure 1d) and were about half as thick as the knots. This corresponds to the expected cylinders of bimolecular diameters and clearly indicates the hydrophobic tail-to-tail arrangement of the amphiphiles (Figure 1f).

The sketch of Figure 1f is similar to models of cholesteric liquid crystals. The crystal planes, which are perturbed by chiral centers,[17] are just replaced by micellar, circular planes. The helical packing of the cylinders of figure 1f can also be seen in analogy to tubular packings of protein spheres in biological microtubules.[18] The hydrophobic core of the micellar cylinder has a diameter of about 20 Å (16 CH_2 groups) and should be encircled by CO–HN hydrogen bonds. The stacking of the molecules in the micellar cylinder is then caused by the space requirements of the oligomethylene chains. That the subtle stereochemical interactions between the chiral centers in the carbohydrate moiety lead to the helices with a 238 Å pitch will be discussed in another paper, which compares the aggregation behavior of eight diastereomeric hexonamides.

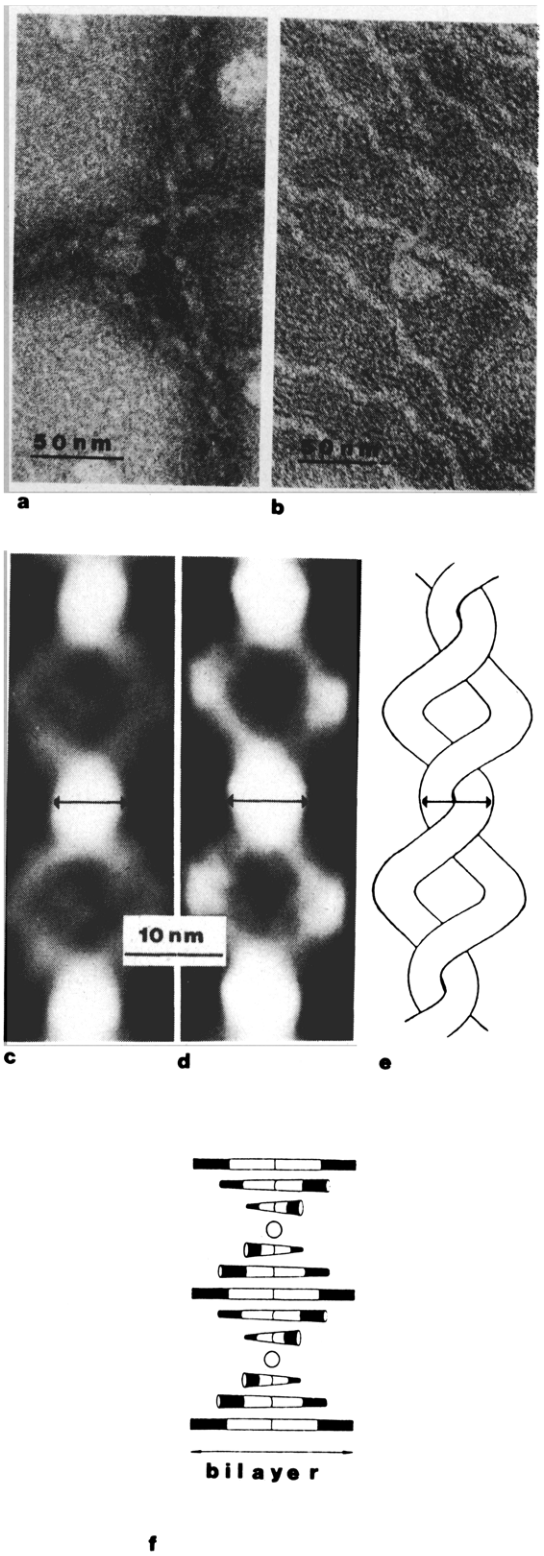


Figure 1. Fibrous aggregates, (a) the bulgy double helix and (b) the twisted ribbon, from **1a**. Electron micrographs, negatively stained. Magnification 56 000 (c, d) computer images of the bulgy helix electron micrographs from **1a** and **1b**. (e, f) Models of the bulgy double helix and the molecular arrangements in the micellar cylinders (see text).

The negatively stained images of the bulgy helices (Figure 1a,c,d) cannot be used to determine the handedness of the helices, since the total projections do not provide clear information of the curbing perpendicular to the plane. Shadowed specimen of the bulgy helices of *N*-octyl-D-gluconamide, however, showed right handedness. The L enantiomer produced left-handed helices (Figure 2a,b). If both enantiomers were mixed in a 1:1 ratio only nonfibrous, nontwisted platelets were found on electron micrographs (Figure 2). This is in agreement with the macroscopic

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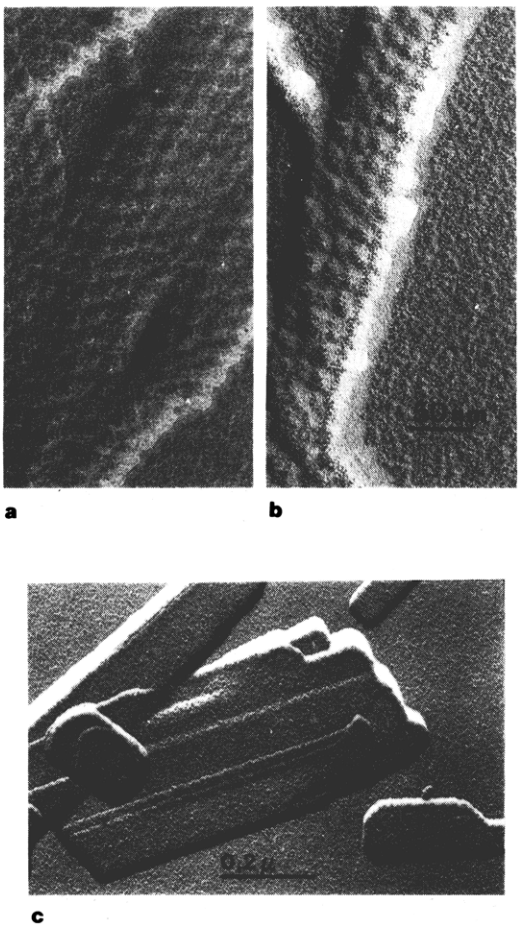

Figure 2. Electron micrographs of the platinum shaded bulgy helices of (a) **1a** and (b) **2** and (c) the racemic **1a** + **2** platelets. Magnification 56 000.

observation that the racemate does not form clear gels. Only white precipitates are formed when the hot solution of the racemate is cooled down. Similar observations have been made with chiral and racemic fatty acid derivatives in organic solvents.[9,10]

The single ropes with irregular undulations reproduced in Figure 1b have a diameter of 70 ± 5 Å, which corresponds to four molecular lengths. They were sometimes observed besides the bulgy helices and are thought to be the first intermediate between the bulgy double helices and the thicker helices of unknown multiplicity in aged gels.[7,16]

Crystal Structures. The anhydrous crystals of **1a** give no evidence for a bilayer structure or a chiral superstructure. The glucose moieties are in an undisturbed *all-trans* conformation and produce planar, hydrogen-bonded sheets. The hydrophobic bilayer of the aqueous gel is replaced by an enantiopolar[19] head-to-tail arrangement of the sheets.[11] This means that half of the molecular sheets turn by 180° on the way from the bulgy helix strands to crystalline material. We assume that this process occurs stepwise. Already in the ropes with four molecular layers the inner layers could be attached head-to-tail to the outer layers which are in contact with water. Comparisons of thermograms of fresh gels, aged gels, and liquid crystals (see next section) will provide evidence for this assumption.

The crystal structure of racemic **1a** + **2** could not be solved so far, because only small crystals were obtained. This difficulty presumably results from the tendency of head-to-head molecular bilayers to retain large amounts of water. Similar difficulties have been observed with racemates of hydrophobic amino acids. Some of these crystal structures are, however, known. In DL-valine and DL-isoleucine crystals it was found, that the L- or D-amino acid

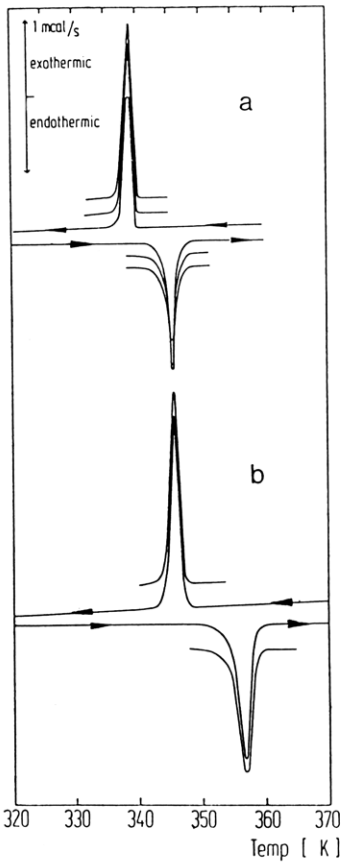

Figure 3. DSC thermograms of (a) helical fibers in gels of **1a** (ΔH = −23 ± 2 kJ/mol) and (b) racemic platelets of **1a** + **2** (ΔH = −32 ± 2 kJ/mol).

sheets were perfectly enantiopolar, whereas the DL-amino acid sheets in the same crystal were apolar bilayers.[20] In DL-tyrosine the pertinent symmetry elements for the coupling of D and L layers are glide planes and in L-tyrosine screw axes.[21] The molecular packing in the racemate is somewhat denser.[21] Racemates of hydrophobic amino acids also are much less soluble in water than the pure enantiomers.[22] As a final example we cite results of experiments with monomolecular layers on water surfaces: racemates occupy in general a 10–20% smaller molecular surface area than pure enantiomers of amphiphiles.[23]

All of these data on crystals and crystallization of chiral molecules with a hydrophobic and a hydrophilic part show that the crystallization of racemates is more favorable than that for pure enantiomers. Only with racemates are the molecular arrangements in hydrophobic aggregates kept in the crystals. This rule, however, is not followed when the polar and apolar parts of the amphiphilic molecules differ very much in width. In these cases interdigitized arrangements will prevail.[24]

Differential Scanning Calorimetry

The rearrangement of bilayer cylinders to enantiopolar crystal planes occurs within hours or days. It should therefore be detectable by thermodynamic measurements.

Thermograms of liquid crystals of dodecyl 1-*O*-α-D-glucopyranoside and similar compounds[25] have been published. The heating curves show two or three peaks between 330 and 370 K, which have been attributed to the disengagement of the hydro-

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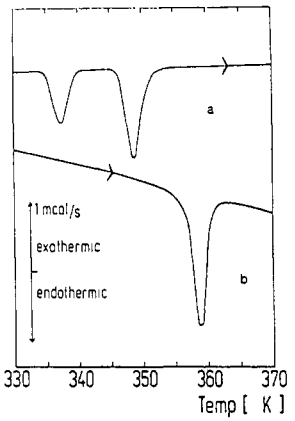

Figure 4. (a, b) DSC thermograms of the aged materials of Figure 3.

carbon chains in the three-dimensional crystal lattice.[26] At 420–430 K the hydrogen-bonded clusters of the liquid crystalline phase were supposed to melt to give an isotropic homogenous liquid.[25] The cooling curve of the dodecylglucoside only reproduced the clearing point at 420–430 K,[25] but no other phase transition was observed. In the case of octyl gluconamide **1a** liquid crystal phase transitions at 345 and 360 and a clearing point of 432 K have been observed.[25,26] Attempts to evaluate structural changes upon heating of crystals by x-ray analysis failed.[11]

We examined vesicular solutions of **1a**. They show a sharp exothermic peak at 339 K on cooling. Supercooling of the solutions has never been observed; gelation occurs reproducibly and almost independent of concentration at 339 K. In the heating curve an equally sharp endothermic peak at 346 K is found for liquefication of the gel (Figure 3a). The racemic platelets show almost identical thermograms with peaks shifted to higher temperatures (Figure 3b). In both cases, the D-enantiomer gel and the racemate platelets, the gelation and liquefication curves were fully reproducible, if the gel was reheated within an hour. Aged gels gave different liquefication thermograms (see below), but on cooling of the hot vesicular solutions always the same gelation thermograms were found.

An important difference between the thermograms of D-enantiomer gel and of the racemic platelets (Figure 4) was observed after ageing of both aggregates. After 8–16 h, when the clear gel had turned white without showing precipitates, it produced two broad peaks in the DSC thermogram at 247 K (ΔH = 30 kJ/mol) and 238 K (ΔH = 17 kJ/mol). The ratio of enthalpies of 1.85 ± 0.1 was independent of the concentration of **1a** within a range of 2–15% (w/v). The DSC thermogram of the aged racemate sludge also showed a broadening of the original peak at 85 °C, but no trace for a second, low-temperature transition was found.

The thermograms displayed in Figure 3 clearly point to closely similar aggregation processes on the ways from spherical vesicles to (a) chiral fibers and (b) racemic platelets. This should be the formation of extended linear amide hydrogen bonds within hydrophobic bilayers. A comparison of the melting curves of aged aggregates in Figure 4, on the other hand, shows that only in the chiral fibers do important molecular rearrangements occur. Both findings fit the model, which is described in the next section.

The Chiral Bilayer Effect

The results described and analogous reports on organogels suggest that (i) only chiral amphiphiles can produce helical fibers and (ii) rearrangement of the bilayer fiber structures to enantiopolar crystal layers is slow. We relate these observations to the fact that regular crystalline arrangements of the substituents of chiral centers determine the aggregate structures. Crystalline

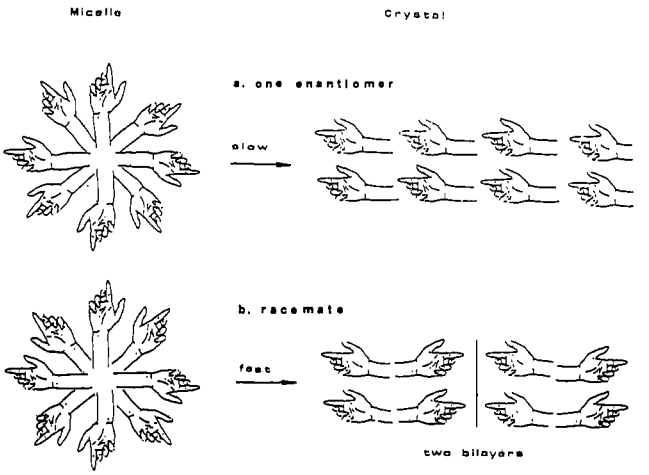

Figure 5. The chiral bilayer effect: (a) chiral micellar cylinders rearrange slowly to enantiopolar crystals; (b) the hydrophobic bilayer of achiral micellar cylinders is retained in the crystal. Crystallization is fast.

regularity in a hydrophobic bilayer asks for screw-symmetric fibers. In a planar crystal layer head-to-tail arrangements of the chiral amphiphiles are preferred, to achieve homogenous packing of all chiral centers. The situation is illustrated in Figure 5 with right hands. In the circular arrangement all thumbs point into the same direction of the turn of the screw and all palms are upside. In the crystal plane thumbs and palms of all right hands occupy identical spaces (Figure 5b). It is obvious from Figure 5a that the screw corresponds to hydrophobic bilayer where all the polar groups (=hands) are in touch with the aqueous environment.

The helical structure must be dehydrated and the molecular sheets must be turned by 180° to precipitate the fibers in the gel as crystals. Both processes are presumably unfavorable in aqueous solution and are accordingly slow. A racemic mixture, on the other hand, has a low tendency to form screw-like bilayers. The hydrophobic bilayer may easily crystallize, if racemic compounds are formed (Figure 5b). The same argument applies, of course, for achiral amphiphiles.

The positive effect of chirality on the lifetime of hydrophobic aggregate fibers and metastable gels is called "chiral bilayer effect". It may well be that similar effects also stabilize gels with chiral polymer fibers.[27] Helices may be converted to sheets without the intermediacy of a coil, when both mirror images are combined. The fibrous aggregates from gluconamides, chiral fatty acids,[10] and guanylic[28] acid may also be used as substrates for condensation reactions in organic and aqueous media. This may provide models for the prebiological formation of biopolymers. In general, chirality may be an important presupposition for the construction of bioorganic organisates. Another challenge is the search for racemic crystals, which dissociate into less soluble chiral fibers. An analogous case has already been reported in chiral monolayers.[8] Work along these lines is in progress.

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Supplementary Material Available: Elemental analysis of **1a–c** and **2** and electron micrographs and spectra of various gluconamide aggregates (15 pages). Ordering information is given on any current masthead page.

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